STUDY TITLE

K32:
Acute Oral Toxicity – Up-And-Down Procedure in Rats

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)
OECD Guidelines for the Testing of Chemicals, Test No. 425 (2008)

AUTHOR

Carolyn Lowe, LATG

STUDY COMPLETED ON

April 18, 2017

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

44534

SPONSOR

Koch Agronomic Services
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

K32

This study meets the requirements of U.S. EPA GLP (TSCA): 40 CFR Part 792, 1989 and OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17, OECD, Paris, 1998. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: Carolyn Lowe Date: 4/18/2017

Name of Signer: Carolyn Lowe, LATG

Name of Company: Product Safety Labs

Sponsor: Koch Agronomic Services Date: 4/18/2017

Name of Signer: Eric A. Searcy

Name of Company: Koch Agronomic Services

Submitter: Eric A. Searcy Date: 4/18/2017

Name of Signer: Eric A. Searcy

Name of Company: Koch Agronomic Services

QUALITY ASSURANCE STATEMENT

The Product Safety Labs' Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Performed By	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	A. Adamiec; M. Zakrzewski	Oct 1, 2013[1]; Feb 20, 2017	Oct 1, 2013; Feb 20, 2017
In-process inspection: *Day 14 in-life observations and body weight for Animal #3104*	A. Adamiec	Feb 8, 2017	Feb 8, 2017
Raw data audit	M. Zakrzewski	Feb 20, 2017	Feb 20, 2017
Draft report review	M. Zakrzewski	Feb 20, 2017	Feb 20, 2017

Final report reviewed by:

Maryann Zakrzewski
Quality Assurance Auditor
Product Safety Labs

April 18, 2017
Date

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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K32: ACUTE ORAL TOXICITY – UP-AND-DOWN PROCEDURE IN RATS

PROTOCOL NO.:	P320.UDP
STUDY NUMBER:	44534
SPONSOR:	Koch Agronomic Services 2883 Miller Rd. Decatur, GA 30035
TEST SUBSTANCE IDENTIFICATION:	K32 Lot #: 55700-30-13
DATE RECEIVED:	December 1, 2016
PSL REFERENCE NO.:	161201-2R
STUDY INITIATION DATE:	December 7, 2016
DATES OF TEST:	January 8 - February 10, 2017
NOTEBOOK NO.:	44534: pages 1-33

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to K32 by the oral route.

2. SUMMARY

An acute oral toxicity test was conducted with rats to determine the potential for K32 to produce toxicity from a single dose via the oral route. Under the conditions of this study, the acute oral LD_{50} of the test substance is greater than 2000 mg/kg of body weight in female rats.

An initial limit dose of 2000 mg/kg was administered to one healthy female rat by oral gavage. Due to the absence of mortality in this animal, four additional females received the same dose level, sequentially. Since these animals survived, no additional animals were tested. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration (initial) and again on Days 7 and 14 (terminal) following dosing. Necropsies were performed on all animals at terminal sacrifice.

All animals survived test substance administration and gained body weight during the study. Following administration, three animals exhibited reduced fecal volume and one animal exhibited soft feces. However, the animals recovered by Day 2 and along with the remaining animal appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

The test substance, identified as K32, Lot #: 55700-30-13, was received on December 1, 2016, and was further identified with PSL Reference Number 161201-2R. The test substance was stored frozen. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: K32 - 97.6%
Water - 2.4%, CAS #7732-18-5

Physical Description: Off-white to pale yellowish gel

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable

B. Animals

3.B.1 Number of Animals: 5

3.B.2 Sex: Female, nulliparous and non-pregnant.

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino.

3.B.4 Age/Body Weight: Young adult (9-12 weeks)/190-214 grams at experimental start.

3.B.5 Source: Received from SAGE® Labs on December 21, 2016 and January 11, 2017.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging, which conforms to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Enrichment (e.g., toy) was placed in each cage. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-23°C and 39-60%, respectively.

4.A.3 Animal Room Air Changes/Hour: 13. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

4.A.4 Photoperiod: 12-hour light/dark cycle

4.A.5 Acclimation Period: 9-28 days

4.A.6 Food: Envigo Teklad Global 16% Protein Rodent Diet® #2016. The diet was available *ad libitum*, except during fasting.

4.A.7 Water: Filtered tap water was supplied *ad libitum*.

4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this

study. Analyses of the food and water are conducted regularly and the records are kept on file at Product Safety Labs.

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number, dose level, identification, and sex of the animal.

4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study number 44534, constituted unique identification.

5. PROCEDURE

A. Selection of Animals

Prior to each dosing, experimentally naive rats were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Five healthy, naive female rats (not previously tested) were selected for test.

B. Preparation of Test Substance

The test substance, as received, was a gel. Preliminary sample preparation assessments conducted by PSL indicated that the test substance was not dosable in distilled water, corn oil, or 0.5% carboxymethylcellulose (CMC), but was found to be dosable in dimethyl sulfoxide. The test substance was administered as a 40% w/w mixture in dimethyl sulfoxide. Preliminary sample preparation assessments conducted by PSL indicated that mixtures in excess of 40% (i.e., 50%) were too viscous to be administered properly. Each preparation was mixed well prior to use.

C. Dose Calculations

Individual doses were calculated based on the initial body weights, taking into account the density (determined by PSL) and concentration of the test mixture.

D. Dosing

The prepared test substance was administered to the stomach using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

Individual animals were dosed as follows:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	2000	S	S
2	3102		S	S
3	3103		S	S
4	3104		S	S
5	3105		S	S

S – Survival

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes approximately 30 minutes post-dosing, during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma.

F. Body Weights

Individual body weights of the animals were recorded prior to test substance administration (initial) and again on Days 7 and 14 (terminal) following dosing.

G. Necropsy

All rats were euthanized via CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

6. STATISTICAL ANALYSIS

Statistical analysis was limited to the calculation of the mean density value for dosing.

7. STUDY CONDUCT

This study was conducted at Product Safety Labs' (PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. The Study Director for this study was Carolyn Lowe, LATG. The primary scientist for this study was Harry Maselli, ALAT, with contributions from Joshua Battaglia, BS and Stephanie De Carlo, BS. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- U.S. EPA GLP: Toxic Substances Control Act (TSCA): 40 CFR Part 792, 1989
- OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17, OECD, Paris, 1998

and based on the following testing guidelines:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)
- OECD Guidelines for the Testing of Chemicals, Test No. 425 (2008)

8. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

9. AMENDMENTS TO THE PROTOCOL

None.

10. DEVIATIONS FROM THE PROTOCOL

None.

11. FINAL REPORT AND RECORDS TO BE MAINTAINED

Information on care of the test system, equipment maintenance and calibration, storage, usage, and disposition of the test substance, and all other records that would demonstrate adherence to the protocol will be maintained. Facility records which are not specific to the subject study will be maintained by the testing facility and archived according to PSL SOP.

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at PSL, is maintained in the PSL Archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by PSL.

12. RESULTS

Individual body weights and doses are presented in Table 1. Individual cage-side and necropsy observations are presented in Tables 2 and 3, respectively.

All animals survived test substance administration and gained body weight during the study. Following administration, three animals exhibited reduced fecal volume and one animal exhibited soft feces. However, the animals recovered by Day 2 and along with the remaining animal appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

13. CONCLUSION

Under the conditions of this study, the acute oral LD_{50} of K32 is greater than 2000 mg/kg of body weight in female rats.

14. REFERENCES

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals (8th ed.)*. Washington, DC: The National Academies Press.

SIGNATURE

K32

I, the undersigned, declare that the methods, results, and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Carolyn Lowe, LATG
Study Director
Product Safety Labs

4/18/2017
Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose[1]
			Initial	Day 7	Day 14	mL
3101	F	2000	214	238	258	0.92
3102	F		190	240	257	0.82
3103	F		190	214	233	0.82
3104	F		196	236	243	0.85
3105	F		199	224	251	0.86

[1] The test substance was administered as a 40% w/w mixture in dimethyl sulfoxide. Density – 1.157 g/mL.

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Observation	Day of Observation (x=observation is present)																
				0(0.5 hr)	0(3 hrs)	0(3.5 hrs)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3101	F	2000	Active and healthy	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Reduced fecal volume				x													
3102	F	2000	Active and healthy	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x
			Soft feces				x													
3103	F	2000	Active and healthy	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
3104	F	2000	Active and healthy	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x
			Reduced fecal volume				x													
3105	F	2000	Active and healthy	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Reduced fecal volume				x													

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Organ / Tissue	Observation
3101	F	2000	All tissues and organs	No gross abnormalities
3102	F	2000	All tissues and organs	No gross abnormalities
3103	F	2000	All tissues and organs	No gross abnormalities
3104	F	2000	All tissues and organs	No gross abnormalities
3105	F	2000	All tissues and organs	No gross abnormalities